Infusion of reconstituted high-density lipoproteins (rHDL) is being studied in clinical trials as an adjunctive therapy for gram-negative sepsis. Since no data are available on its possible effects in systemic candidiasis, we investigated the effect of rHDL infusion into volunteers on the growth of Candida albicans. C. albicans growth was 10- to 100-fold higher in the plasma of volunteers infused with 80 or 100 mg/kg rHDL than in plasma collected before infusion; administration of 60 mg/kg rHDL had marginal effects. In vitro, the isolated lipoprotein subfractions had a growth-promoting effect on C. albicans. These data suggest potential adverse effects of rHDL if infused into patients with systemic candidiasis. Thus, rHDL infusion into patients with sepsis caused by an unknown microorganism may be contraindicated.

Materials and Methods

Infusion of rHDL into human volunteers. Three groups of four healthy volunteers each were infused with rHDL at various doses (60, 80, or 100 mg/kg) over a 4-hour period. Blood samples were collected just before the infusion (time 0) and 4, 12, and 24 hours after the start of the infusion. Plasma samples from subjects receiving the same dose were pooled at each time point and were provided by Dr. Jan Eva Doran and Dr. Alphonse Hubsch (ZLB Central Laboratory, Bern, Switzerland).

Isolation of the lipoprotein subclasses. LPS-free very-low-density lipoprotein (VLDL; final cholesterol concentration, 0.9 mmol/L), LDL (2.0 mmol/L cholesterol), and HDL (0.5 mmol/L cholesterol) subclasses, as well as lipoprotein-depleted plasma (LPDP) (<0.1 mmol/L cholesterol), were isolated by sequential ultracentrifugation from fresh EDTA-treated plasma of healthy volunteers who did not receive rHDL infusion. The methods for isolation of lipoprotein subclasses have been described earlier [10]. Lipoproteins were dialyzed for 24 hours against 0.05 mM phosphate buffer, pH 7.4, containing 5 mM EDTA/L, with one exchange of the buffer. Lipoprotein subfractions isolated from six volunteers were studied in triplicate.
**Results**

**Growth of C. albicans in plasma of volunteers infused with rHDL.** The total cholesterol and HDL concentrations in the group receiving 60 mg/kg rHDL increased from a basal level of 3.87 and 0.95 mmol/L, respectively, to 4.63 and 1.03 mmol/L at the end of the 4-hour infusion interval to 4.72 and 1.32 mmol/L at 12 hours and 4.30 and 1.58 mmol/L at 24 hours after the start of the infusion. The total cholesterol and HDL concentrations in the group receiving 100 mg/kg rHDL increased from 3.58 and 1.12 mmol/L, respectively, to 4.38 and 1.30 mmol/L at the end of the 4-hour infusion interval and to 5.00 and 1.60 mmol/L at 12 hours and 4.50 and 2.33 mmol/L at 24 hours after the start of the infusion.

The control growth is represented by the growth of *C. albicans* in plasma obtained from volunteers before the start of rHDL infusion (time 0; figure 1). Infusion of 60 mg/kg rHDL had little effect on the growth of *C. albicans*, but *C. albicans* grew better in plasma obtained after infusion of 80 or 100 mg/kg rHDL (figure 1). The number of *C. albicans* colony-forming units grown in plasma obtained 24 hours after rHDL infusion was ~100-fold greater than that grown in control plasma. Similarly, infusion of 100 mg/kg rHDL at various infusion rates (4–12 hours) resulted in a significantly increased outgrowth of *C. albicans*, compared with the outgrowth of the yeast in plasma obtained before infusion (P < .05, Kruskal-Wallis test; data not shown).

**Growth of C. albicans in freshly isolated human lipoprotein subclasses.** As shown in figure 2A, *C. albicans* grew better in the lipoprotein emulsions from normal human donors than in lipoprotein-depleted plasma, suggesting that all lipoprotein subfractions are able to either enhance *C. albicans* growth or to inactivate fungistatic factors present in plasma. To further investigate these two possibilities, the growth of *C. albicans* was investigated in plasma of LDLR−/− mice.

**Growth of C. albicans in plasma from hyperlipoproteinemic LDLR−/− mice.** In contrast to plasma collected from human volunteers, murine plasma inhibited the growth of *C. albicans* in vitro. However, this growth inhibition was significantly reduced in plasma from LDLR−/− mice [6]. After 12 hours of incubation of 10^4 cfu of *C. albicans* in LDLR−/− plasma, the log colony count ± SD was 3.7 ± 1.1, versus 3.2 ± 0.9 in control plasma, and after 24 hours of incubation, it was 3.4 ± 1.3, versus 3.0 ± 0.7 in control plasma (P < .05). To discern whether this effect is due to the use of lipoproteins as nutrients or to the binding and neutralization of a candidacidal factor by lipoproteins, we compared the growth curves of *C. albicans* in lipoprotein-depleted plasma from hyperlipoproteinemic and normal mice. *C. albicans* grew significantly better in lipoprotein-depleted plasma from LDLR−/− mice than in lipoprotein-depleted plasma from the LDLR+/+ controls, although both subfractions contained similar amounts of lipoproteins (<0.01 mmol/L cholesterol) after the depletion procedure (figure 2B). This suggests that a candidacidal factor may be bound to lipo-
tein subfractions and in hyperlipoproteinemic plasma from LDLR−/− mice. These data are in agreement with our previous findings in hyperlipoproteinemic LDLR−/− mice, which were more susceptible to disseminated candidiasis because of an increased fungal outgrowth in their organs [6]. Although lipid profiles differ between mice and humans, the results of both studies suggest that hyperlipidemia can have deleterious effects by stimulating C. albicans growth in both species.

At least two different mechanisms seem to be responsible for the observed effects. First, in agreement with other reports showing increased growth of C. albicans in lipid emulsions used for parenteral feeding [7, 8], lipids themselves seem to promote the growth of Candida. Cholesterol may act as a nutrient for C. albicans, as has been demonstrated for other microorganisms, such as Staphylococcus aureus [12]. After infusion of rHDL, natural HDL has to be formed through extraction of cholesterol from cell membranes and other lipoprotein subfractions, since rHDL consists of phospholipids and recombinant apolipoprotein A-1 without cholesterol [2]. This process requires several hours, and this explains the increasing effect of rHDL on C. albicans growth at later time points after infusion. Indeed, the total cholesterol and HDL concentrations were found to be higher 12 and 24 hours after the start of the infusion than at the end of the 4-hour infusion interval.

Second, since the growth of C. albicans was enhanced in lipoprotein-depleted plasma of hyperlipoproteinemic mice, binding and neutralization of candidacidal factors, such as sphingosine [13], platelet microbicidal protein [14], or the calprotectin complex [15], by lipoproteins may contribute to the observed effect. Although no lipoproteins were present in lipoprotein-depleted plasma isolated from either LDLR−/− or normal mice, C. albicans growth was significantly better in LPDP from mice lacking LDL receptors (●) than in that from control mice (■). Each point represents mean ± SD of 5 experiments. * P < .05 (Kruskal-Wallis test).

Figure 2. In vitro growth of Candida albicans in the presence of lipoproteins. A, C. albicans grows better in plasma containing freshly isolated very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL), diluted 1:1 in Sabouraud broth, than in lipoprotein-depleted plasma (LPDP). B, C. albicans grows significantly better in LPDP from mice lacking LDL receptors (●) than in that from control mice (■). Each point represents mean ± SD of 5 experiments. * P < .05 (Kruskal-Wallis test).

proteins and extracted together with lipoproteins during the lipoprotein depletion procedure.

Discussion

The results of the present study show that rHDL infusion (at ≥80 mg/kg) increases the growth of C. albicans in the plasma of human volunteers by 10- to 100-fold, compared with the basal growth curve in their plasma collected before rHDL administration. In contrast, there was no significant difference in the growth of C. albicans in plasma before and after infusion of a moderate amount of rHDL (60 mg/kg). Similarly, C. albicans growth was increased in the presence of freshly isolated human lipopro-
tentiates also the growth of other microorganisms, such as *S. aureus* [7–9], and the clinical implication of this phenomenon for rHDL infusion warrants further investigation.

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**References**